



**Whole Effluent Toxicity Test Report:
Washington Beef LLC.**

June 2014

Report date: June 30, 2014

Submitted to:

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1.0 INTRODUCTION

A whole effluent toxicity test was conducted using effluent samples collected from the Washington Beef LLC wastewater treatment plant in June 2014. A chronic bioassay was conducted using the test organism *Ceriodaphnia dubia* (*Ceriodaphnia*). Testing was performed at Rainier Environmental Laboratory located in Tacoma, Washington.

2.0 METHODS

2.1 Sample Collection and Transport

Effluent samples were collected into 4-liter (L) LDPE cubitainers by Washington Beef personnel. The samples were packed in coolers containing ice and shipped to Rainier Environmental by overnight delivery service. Appropriate chain-of-custody procedures were employed during collection and transport (Appendix D).

2.2 Sample Receipt

Upon arrival at the laboratory, coolers were opened, samples inspected, and the contents verified against information provided on the chain-of-custody forms. Receipt temperature was measured and recorded on the chain-of-custody form. The standard water quality parameters were measured and recorded on sample check-in sheets (Appendix B). Samples were stored at 4°C in the dark until used for testing.

2.3 Test Methods

A chronic toxicity test was conducted according to procedures presented by USEPA (2002). The methods are summarized in Table 1. The procedure involved a 7-day static-renewal exposure to the effluent. The endpoints from these tests were *Ceriodaphnia* survival at the end of exposure and reproduction at test termination or production of 3 broods, whichever occurred first. Termination of the test occurred when at least 60 percent of surviving control females produced 3 broods. The test was ended on Day 7.

Table 1. Summary of methods for the 7-day *Ceriodaphnia* survival and reproduction test.

Test initiation date and time	6/3/14; 1415h
Test termination date and time	6/10/14; 1500h
Test organism	<i>Ceriodaphnia dubia</i>
Test organism source	In-house cultures
Test organism age	< 24 hours
Test duration	7 days; Test terminated when 60% of controls reached 3 broods
Feeding	1:1 mixture YTC:algal suspension daily
Test chamber; test solution volume	30 mL plastic cup; 15 mL
Test temperature	25 ± 1°C
Dilution water	Diluted mineral water
Test concentrations (% sample)	100, 50, 25, 12.5, 6.25, laboratory control
Number of organisms/chamber	1
Number of replicates	10
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test protocol	EPA-821-R-02-013
Test acceptability criteria for controls	≥ 80% survival; ≥ 15 neonates per surviving adult
Reference toxicant	Sodium chloride

3.0 RESULTS AND DISCUSSION

Details of standard water quality measurements conducted upon receipt of samples are provided in Table 2.

Table 2. Final Effluent sample information.

Parameter	WET	
Rainier Log-in No.	14-056	14-058
Collection date and time	6/2/2014; 0800h	6/4/2014; 0725h
Receipt date and time	6/3/2014; 1020h	6/5/2014; 1205h
Receipt temperature (°C)	1.2	1.5
Dissolved oxygen (mg/L)	5.7	5.4
pH	8.04	8.02
Conductivity (µS/cm)	5120	4390
Salinity (ppt)	2.7	2.3
Hardness (mg/L CaCO ₃)	164	180
Alkalinity (mg/L CaCO ₃)	160	132
Total Chlorine (mg/L) ^a	<0.03	<0.03
Total Ammonia (mg/L) ^b	<1.0	<1.0

^{a,b} See reference below

Note: Total chlorine and ammonia values are measured by Rainier Environmental to provide additional information in support of the bioassay test procedures. They are not intended to be interpreted as exact values, particularly near the detection limits where interferences are most likely to become apparent.

^a Total chlorine is measured using a Hach DR/2000 spectrophotometer and colorimetric DPD Total Chlorine Reagent. Under optimum conditions, the method has a range of 0.03 to 2.0 mg/L \pm 0.01 mg/L total chlorine. Compounds in the sample that interfere with chlorine detection include bromine, manganese, chromium, ozone, and peroxides. Additional interferences include extreme pH values and high alkalinity (greater than 300 mg/L Ca CO₃).

^b Total ammonia is measured using a Hach DR/2000 spectrophotometer following the salicylate method which uses AmVer Diluent Reagent Test 'N' Tube kits. Under optimum conditions, the method has a range of 0.4 to 50.0 \pm 0.1 mg/L NH₃-N. High sample turbidity will give erroneously high values. Additional interferences to the method include extreme pH and high concentrations of magnesium, iron, nitrite, nitrate, or sulfate.

Results for the toxicity tests are summarized in Table 3. Individual statistical summaries for the test and copies of the laboratory bench sheets are provided in the Appendices A-D.

The NOEC (concentration at which no effect on the organisms is detected) was 50 percent sample for survival and <6.25 percent for reproduction. The associated chronic toxicity unit (TUC; 100 percent sample divided by the NOEC) was 2 for survival and >16 for reproduction.

Table 3. Summary of toxicity test results.

Sample	Endpoint	NOEC (% effluent)	Chronic Toxicity Unit (TUC) ^a
Final Effluent	Survival	50	2.0
	7-day Reproduction	<6.25	>16

^a Chronic toxicity unit (TUC = 100 \div NOEC)

4.0 QA/QC

Samples were received in good condition and within the temperature range specified by EPA (2002). The third sample never arrived. The second sample was used instead of the missing sample. The toxicity tests met all acceptability criteria for performance of control organisms. There were no deviations from protocol and water quality parameters remained within the ranges specified in the corresponding test methods throughout the tests.

Results for the most recent reference toxicant test used to monitor laboratory performance and test organism sensitivity are summarized in Table 4 and Appendix C. The coefficients of variation (CV) for the endpoints are also shown in the table. The results for the reference toxicant test fell within the acceptable range of mean \pm two standard deviations of historical test results indicating that the test organisms were of an appropriate degree of sensitivity.

Table 4. Reference toxicant test results.

Species	Endpoint	Date initiated	LC ₅₀ /EC ₅₀	Acceptable Range	CV (%)
<i>Ceriodaphnia</i>	7d survival	5/20/2014	1.74 g/L NaCl	1.26 – 2.62 g/L	20.0
	7d reproduction	5/20/2014	1.55 g/L NaCl	1.08 – 1.60 g/L	10.4

REFERENCES

Tidepool Scientific Software. 2001-2011. CETIS Comprehensive Environmental Toxicity Information System Software, Version 1.8.4.6.

USEPA. 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. EPA-821-R-02-013. pp. 141-196.

Appendix A
Statistical Summaries and Raw Bench Sheets

CETIS Summary Report

 Report Date: 30 Jun-14 09:26 (p 1 of 2)
 Test Code: 1406-019 | 05-7351-3589

Ceriodaphnia 7-d Survival and Reproduction Test
Rainier Environmental Laboratory

Batch ID:	14-9017-3753	Test Type:	Reproduction-Survival (7d)	Analyst:	Eric Tollefson
Start Date:	03 Jun-14 14:15	Protocol:	EPA/821/R-02-013 (2002)	Diluent:	Perrier Water
Ending Date:	10 Jun-14 15:00	Species:	Ceriodaphnia dubia	Brine:	
Duration:	7d 1h	Source:	In-House Culture	Age:	<24h
Sample ID:	02-4421-9928	Code:	14-056	Client:	Washington Beef
Sample Date:	02 Jun-14 08:00	Material:	POTW Effluent	Project:	
Receive Date:	03 Jun-14 10:20	Source:	Washington Beef (WA0050202)		
Sample Age:	30h (1.2 °C)	Station:	Outfall 002		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
17-4965-1067	7d Survival Rate	50	100	70.71	NA	2	Fisher Exact/Bonferroni-Holm Test
06-6148-7544	Reproduction	<6.25	6.25	NA	26.0%	>16	Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
02-1599-5751	7d Survival Rate	LC5	0.9354	0.4862	8.893	106.9	Linear Interpolation (ICPIN)
		LC10	2.746	1.209	51.69	36.42	
		LC15	50	2.282	55.23	2	
		LC20	53.27	3.878	59.5	1.877	
		LC25	56.75	6.25	66.03	1.762	
		LC40	68.58	59.5	100	1.458	
		LC50	77.78	65.33	N/A	1.286	
06-3326-2771	Reproduction	IC5	0.3274	0.2206	1.165	305.4	Linear Interpolation (ICPIN)
		IC10	0.7621	0.4898	3.689	131.2	
		IC15	1.339	0.8185	6.863	74.68	
		IC20	2.105	1.22	8.329	47.51	
		IC25	3.122	1.709	10.45	32.03	
		IC40	9.901	3.927	52.95	10.1	
		IC50	54.49	7.6	59.39	1.835	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
02-1599-5751	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
17-4965-1067	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
06-3326-2771	Reproduction	Control Resp	16.3	15 - NL	Yes	Passes Acceptability Criteria
06-6148-7544	Reproduction	Control Resp	16.3	15 - NL	Yes	Passes Acceptability Criteria
06-6148-7544	Reproduction	PMSD	0.2597	0.13 - 0.47	Yes	Passes Acceptability Criteria

7d Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	1	1	1	1	1	0	0	0.0%	0.0%
6.25		10	0.8	0.6426	0.9574	0	1	0.1333	0.4216	52.7%	20.0%
12.5		10	0.7	0.5196	0.8804	0	1	0.1528	0.483	69.01%	30.0%
25		10	0.9	0.7819	1	0	1	0.1	0.3162	35.14%	10.0%
50		10	1	1	1	1	1	0	0	0.0%	0.0%
100		10	0.3	0.1196	0.4804	0	1	0.1528	0.483	161.0%	70.0%

Reproduction Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	16.3	14.63	17.97	9	25	1.415	4.473	27.44%	0.0%
6.25		10	10.6	8.472	12.73	0	15	1.802	5.7	53.77%	34.97%
12.5		10	8.1	5.941	10.26	0	15	1.828	5.782	71.38%	50.31%
25		10	10.6	9.292	11.91	3	15	1.108	3.502	33.04%	34.97%
50		10	9.2	8.595	9.805	7	12	0.5121	1.619	17.6%	43.56%
100		10	0.7	0.2006	1.199	0	4	0.423	1.337	191.1%	95.71%

CETIS Summary Report

Report Date: 30 Jun-14 09:26 (p 2 of 2)
 Test Code: 1406-019 | 05-7351-3589

Ceriodaphnia 7-d Survival and Reproduction Test**Rainier Environmental Laboratory****7d Survival Rate Detail**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1	1	1	1	1	1	1	1	1	1
6.25		1	1	0	1	1	1	1	1	1	0
12.5		1	1	1	1	0	0	1	1	0	1
25		1	1	1	1	1	1	1	1	0	1
50		1	1	1	1	1	1	1	1	1	1
100		0	1	0	0	0	1	0	0	0	1

Reproduction Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	25	15	15	13	9	17	15	17	15	22
6.25		15	13	0	12	15	14	12	13	12	0
12.5		12	15	11	7	0	0	13	14	2	7
25		3	11	11	14	15	13	10	12	10	7
50		12	9	9	7	12	9	8	9	9	8
100		0	1	0	0	0	4	0	0	0	2

7d Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
6.25		1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1
12.5		1/1	1/1	1/1	1/1	0/1	0/1	1/1	1/1	0/1	1/1
25		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1
50		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
100		0/1	1/1	0/1	0/1	0/1	1/1	0/1	0/1	0/1	1/1

Rainier Environmental
Washington Laboratory

Client: Washington Beef
 Sample ID: WEP
 Test No: 1406-019
 Log-In#: 14-056 14-058

Initial and Final Chemistries

Seven Day Chronic Freshwater Bioassay

Start Date & Time: 6/3/14 1415

Stop Date & Time: 6/10/14 1500

Test Species: Ceriodaphnia dubia

Conc. or % CON	Days													
	0		1		2		3		4		5			
	init.	final												
pH	7.22	8.12	7.35	8.01	7.41	8.01	7.22	7.91	7.27	8.03	7.31	8.01	7.22	8.03
DO (mg/l)	7.5	8.2	8.1	8.0	7.9	8.2	7.9	8.2	7.9	8.1	8.0	8.2	8.1	8.2
Cond. (μmhos-cm)	197	208	195	212	195	199	195	211	195	207	195	202	195	205
Temperature (°C)	24.4	25.1	25.1	25.2	24.9	25.2	24.4	25.3	24.3	25.2	25.2	25.3	25.1	25.2
6.25	Days													
	0		1		2		3		4		5			
	init.	final												
pH	7.21	8.06	7.35	8.11	7.42	9.02	7.25	7.92	7.25	8.11	7.31	8.03	7.23	7.95
DO (mg/l)	7.1	8.1	8.0	8.0	7.8	8.2	8.1	8.2	8.1	8.1	8.1	8.2	8.1	8.2
Cond. (μmhos-cm)	566	580	565	583	559	565	560	563	555	562	561	561	561	566
Temperature (°C)	24.4	25.1	25.0	25.2	24.9	25.2	24.5	25.3	24.2	25.4	25.1	25.3	25.1	25.2
12.5	Days													
	0		1		2		3		4		5			
	init.	final												
pH	7.33	8.09	7.37	8.09	7.42	8.12	7.32	7.97	7.34	8.12	7.35	8.04	7.34	7.99
DO (mg/l)	7.2	8.1	8.0	8.1	8.0	8.2	8.1	8.2	8.1	8.2	8.1	8.2	8.1	8.2
Cond. (μmhos-cm)	926	914	919	921	922	927	915	912	912	921	923	927	921	923
Temperature (°C)	24.4	25.0	25.0	25.2	24.9	25.2	24.5	25.3	24.3	25.4	25.1	25.3	25.1	25.2
25	Days													
	0		1		2		3		4		5			
	init.	final												
pH	7.45	8.07	7.42	8.10	7.43	8.09	7.42	8.03	7.37	8.05	7.39	8.04	7.42	8.02
DO (mg/l)	7.3	8.1	8.0	8.1	8.1	8.1	8.2	8.2	8.1	8.2	8.0	8.2	8.0	8.2
Cond. (μmhos-cm)	1592	1588	1576	1573	1567	1571	1531	1529	1527	1534	1579	1584	1569	1566
Temperature (°C)	25.2	25.0	24.9	25.2	24.8	25.2	24.7	25.3	24.3	25.4	25.1	25.3	25.3	25.2
50	Days													
	0		1		2		3		4		5			
	init.	final												
pH	7.51	8.10	7.55	8.12	7.49	8.11	7.54	8.08	7.39	8.17	7.41	8.13	7.52	8.01
DO (mg/l)	6.8	8.1	7.5	8.2	8.0	8.1	8.1	8.2	8.0	8.2	8.1	8.2	8.1	8.2
Cond. (μmhos-cm)	2922	2877	2813	2892	2811	2805	2771	2775	2769	2813	2789	2785	2698	2695
Temperature (°C)	25.2	25.0	24.8	25.2	25.2	25.2	25.1	25.3	24.7	25.4	25.2	25.3	25.4	25.2
100	Days													
	0		1		2		3		4		5			
	init.	final												
pH	7.59	8.18	7.64	8.20	7.61	8.13	7.51	8.21	7.43	8.22	7.47	8.19	7.57	8.12
DO (mg/l)	6.5	8.1	7.1	8.2	7.2	8.1	7.1	8.2	8.1	8.2	8.1	8.2	8.0	8.2
Cond. (μmhos-cm)	5310	5390	5290	5410	5310	5300	5010	5030	4990	5070	5030	5020	5010	5020
Temperature (°C)	25.1	25.0	24.6	25.2	25.5	25.3	25.4	25.3	25.1	25.4	25.2	25.3	25.7	25.2
Tech. Initials	WT	WT	WT											

Dilution Water Batch #: VWR DMW 005
 Test Chamber: VWR

QA Check: WT

Sample Description: Brown
 Animal Source: In House Culture
 Comments: _____ Date Received: _____ Date of Hatch: _____

Ceriodaphnia 7-Day Chronic Survival and Reproduction

Start Date and Time: 6/3/14 1415
Stop Date and Time: 6/10/14 1500

Client/Sample ID: Washington Beef Producers
Test Number: 14062019

Rep	Daily Reproduction								Day 6 Total	Third Brood
	Conc.	Cont	2	3	4	5	6	7		
1	CON	1	-	-	-	-	3	10	13	25
2		38	1	-	-	-	2	1	2	2
3		37	1	-	-	-	2	1	5	15
4		35	1	-	-	-	1	1	3	3
5		35	1	-	-	-	1	1	3	3
6		44	1	-	-	-	1	1	4	4
7		36	1	-	-	-	2	1	6	6
8		60	1	-	-	-	2	1	10	15
9		3	1	-	-	-	2	1	6	6
10		19	1	-	-	-	3	7	10	22
Analyst		St								
Time		1415	1115	1140	1030	1310	1000	1500		
Selen #		003	003	003	003	003	003	003	Day 6 Total	Third Brood
Rep	Conc.	Cont	2	3	4	5	6	8		
1	6.25	30	-	-	-	2	4	9	6	15
2		37	1	-	-	2	1	4	7	13
3		55	1	-	X	1	2	1	0	0
4		35	1	-	-	2	1	6	7	12
5		40	1	-	-	2	1	6	5	11
6		10	1	-	2	1	3	7	14	6
7		47	1	-	-	2	1	6	7	12
8		30	1	-	-	2	1	6	6	12
9		22	1	-	-	2	1	6	5	10
10		33	1	-	X	1	2	1	0	0
									Day 6 Total	Third Brood
Rep	Conc.	Cont	2	3	4	5	6	7	8	
1	2.5	59	-	-	-	2	3	1	1	1
2		9	1	-	-	2	1	4	1	1
3		4	1	-	1	1	2	1	1	1
4		58	1	-	-	2	1	4	1	1
5		54	1	-	X	1	2	1	1	1
6		7	1	-	-	2	1	4	1	1
7		56	1	-	-	2	1	4	1	1
8		2	1	-	2	1	4	1	1	1
9		45	1	-	-	2	1	4	1	1
10		16	1	-	2	1	4	1	1	1
									Day 6 Total	Third Brood
Rep	Conc.	Cont	2	3	4	5	6	7	8	
1	100	38	-	X	-	-	-	-	0	0
2		24	-	-	-	-	-	-	1	1
3		21	1	-	X	-	-	-	1	1
4		15	1	-	1	X	-	-	1	1
5		15	1	-	1	X	-	-	1	1
6		17	1	-	1	X	-	-	1	1
7		7	1	-	2	1	4	1	1	1
8		7	1	-	2	1	4	1	1	1
9		9	1	-	2	1	4	1	1	1
10		51	1	-	2	1	4	1	1	1
									Day 6 Total	Third Brood
Rep	Conc.	Cont	2	3	4	5	6	7	8	
1	100	38	-	X	-	-	-	-	0	0
2		24	-	-	-	-	-	-	1	1
3		21	1	-	X	-	-	-	1	1
4		15	1	-	1	X	-	-	1	1
5		15	1	-	1	X	-	-	1	1
6		17	1	-	1	X	-	-	1	1
7		7	1	-	2	1	4	1	1	1
8		7	1	-	2	1	4	1	1	1
9		9	1	-	2	1	4	1	1	1
10		51	1	-	2	1	4	1	1	1
									Day 6 Total	Third Brood
Rep	Conc.	Cont	2	3	4	5	6	7	8	
1	100	38	-	X	-	-	-	-	0	0
2		24	-	-	-	-	-	-	1	1
3		21	1	-	X	-	-	-	1	1
4		15	1	-	1	X	-	-	1	1
5		15	1	-	1	X	-	-	1	1
6		17	1	-	1	X	-	-	1	1
7		7	1	-	2	1	4	1	1	1
8		7	1	-	2	1	4	1	1	1
9		9	1	-	2	1	4	1	1	1
10		51	1	-	2	1	4	1	1	1
									Day 6 Total	Third Brood
Rep	Conc.	Cont	2	3	4	5	6	7	8	
1	100	38	-	X	-	-	-	-	0	0
2		24	-	-	-	-	-	-	1	1
3		21	1	-	X	-	-	-	1	1
4		15	1	-	1	X	-	-	1	1
5		15	1	-	1	X	-	-	1	1
6		17	1	-	1	X	-	-	1	1
7		7	1	-	2	1	4	1	1	1
8		7	1	-	2	1	4	1	1	1
9		9	1	-	2	1	4	1	1	1
10		51	1	-	2	1	4	1	1	1
									Day 6 Total	Third Brood
Rep	Conc.	Cont	2	3	4	5	6	7	8	
1	100	38	-	X	-	-	-	-	0	0
2		24	-	-	-	-	-	-	1	1
3		21	1	-	X	-	-	-	1	1
4		15	1	-	1	X	-	-	1	1
5		15	1	-	1	X	-	-	1	1
6		17	1	-	1	X	-	-	1	1
7		7	1	-	2	1	4	1	1	1
8		7	1	-	2	1	4	1	1	1
9		9	1	-	2	1	4	1	1	1
10		51	1	-	2	1	4	1	1	1
									Day 6 Total	Third Brood
Rep	Conc.	Cont	2	3	4	5	6	7	8	
1	100	38	-	X	-	-	-	-	0	0
2		24	-	-	-	-	-	-	1	1
3		21	1	-	X	-	-	-	1	1
4		15	1	-	1	X	-	-	1	1
5		15	1	-	1	X	-	-	1	1
6		17	1	-	1	X	-	-	1	1
7		7	1	-	2	1	4	1	1	1
8		7	1	-	2	1	4	1	1	1
9		9	1	-	2	1	4	1	1	1
10		51	1	-	2	1	4	1	1	1
									Day 6 Total	Third Brood
Rep	Conc.	Cont	2	3	4	5	6	7	8	
1	100	38	-	X	-	-	-	-	0	0
2		24	-	-	-	-	-	-	1	1
3		21	1	-	X	-	-	-	1	1
4		15	1	-	1	X	-	-	1	1
5		15	1	-	1	X	-	-	1	1
6		17	1	-	1	X	-	-	1	1
7		7	1	-	2	1	4	1	1	1
8		7	1	-	2	1	4	1	1	1
9		9	1	-	2	1	4	1	1	1
10		51	1	-	2	1	4	1	1	1
									Day 6 Total	Third Brood
Rep	Conc.	Cont	2	3	4	5	6	7	8	
1	100	38	-	X	-	-	-	-	0	0
2		24	-	-	-	-	-	-	1	1
3		21	1	-	X	-	-	-	1	1
4		15	1	-	1	X	-	-	1	1
5		15	1	-	1	X	-	-	1	1
6		17	1	-	1	X	-	-	1	1
7		7	1	-	2	1	4	1	1	1
8		7	1	-	2	1	4	1	1	1
9		9	1	-	2	1	4	1	1	1
10		51	1	-	2	1	4	1	1	1
									Day 6 Total	Third Brood
Rep	Conc.	Cont	2	3	4	5	6	7	8	
1	100	38	-	X	-	-	-	-	0	0
2		24	-	-	-	-	-	-	1	1
3		21	1	-	X	-	-	-	1	1
4		15	1	-	1	X	-	-	1	1
5		15	1	-	1	X	-	-	1	1
6		17	1	-	1	X	-	-	1	1
7		7	1	-	2	1	4	1	1	1
8		7	1	-	2	1	4	1	1	1
9		9	1	-	2	1	4	1	1	1
10		51	1	-	2	1	4	1	1	1
									Day 6 Total	Third Brood
Rep	Conc.	Cont	2	3	4	5	6	7	8	
1	100	38	-	X	-	-	-	-	0	0
2		24	-	-	-	-	-	-	1	1
3		21	1	-	X	-	-	-	1	1
4		15	1	-	1	X	-	-	1	1
5		15	1	-	1	X	-	-	1	1
6		17	1	-	1	X	-			

Appendix B
Sample Check-In Sheets

Rainier Environmental
5013 Pacific Hwy East, Ste. 20
Tacoma, WA 98424

Sample Check-In Information

Client: Washington Boof

Tests Performed: Cd-C
Test ID No(s): 1406-019

Sample Description:
Bone

Sample ID:	<u>WET</u>	<u>WET</u>	
Log-in No. (10-xxxx):	<u>14-056</u>	<u>14-058</u>	
Sample Collection Date & Time:	<u>6/2/14 800</u>	<u>6/4/14 725</u>	
Sample Receipt Date & Time:	<u>6/3/14 1000</u>	<u>6/5/14 1005</u>	
Check-in Temperature (°C)	<u>1.2</u>	<u>1.5</u>	
Temperature OK?	<input checked="" type="radio"/> Y	<input type="radio"/> N	
DO (mg/L)	<u>5.7</u>	<u>5.4</u>	
pH (units)	<u>8.04</u>	<u>8.02</u>	
Conductivity ($\mu\text{S}/\text{cm}$)	<u>5120</u>	<u>4390</u>	
Salinity (ppt)	<u>2.7</u>	<u>2.3</u>	
Tit. Vol / Sam. Vol. / Alkalinity (mg/L)*	<u>4.0 / 35 / 160</u>	<u>3.8 / 25 / 132</u>	<input type="checkbox"/> Filtration? <input checked="" type="checkbox"/> Y <input type="radio"/> N
Tit. Vol. / Sam. Vol. / Hardness (mg/L)* ^a	<u>4.1 / 25 / 164</u>	<u>4.5 / 25 / 170</u>	<input type="checkbox"/> Pore Size: _____
Total Chlorine (mg/L)	<u><0.03</u>	<u><0.03</u>	<input type="checkbox"/> Organisms or Debris
Total Ammonia (mg/L)	<u><1.0</u>	<u><1.0</u>	
Technician Initials	<u>GT</u>	<u>GT</u>	

* = mg/L as CaCO_3 , ^a = Measured for freshwater samples only, NA = Not Applicable,

NM = Not Measured

Freshwater Tests:

Control/Dilution Water Source: test type: cd-c 8:2 (DMW) MHW Other: _____ Alkalinity: 64 Hardness: 80

Control/Dilution Water Source: test type: 8:2 (DMW) MHW Other: _____ Alkalinity: _____ Hardness: _____

Additional Control? Y N = _____ Alkalinity: _____ Hardness: _____

Marine Tests:

Control/Dilution Water Source: test type: ART SW NAT SW Alkalinity: _____ Salinity: _____

Control/Dilution Water Source: test type: ART SW NAT SW Alkalinity: _____ Salinity: _____ Sub-samples for additional chemistry:

Additional Control? Y N = _____ Alkalinity: _____ Salinity: _____

Sample Salted w/ artificial salt? Y N If yes, what ppt? _____ test type: _____

Sample salted w/brine? Y N If yes, what ppt? _____ test type: _____

Comments: Temperature for grab sample must be 0-20°C if received within 1 hour of collection time, 0-12°C if effluent received within 4 hours of collection time, and 0-6°C for all other samples.

QC Check: GT

Appendix C
Reference Toxicant Test

CETIS QC Plot

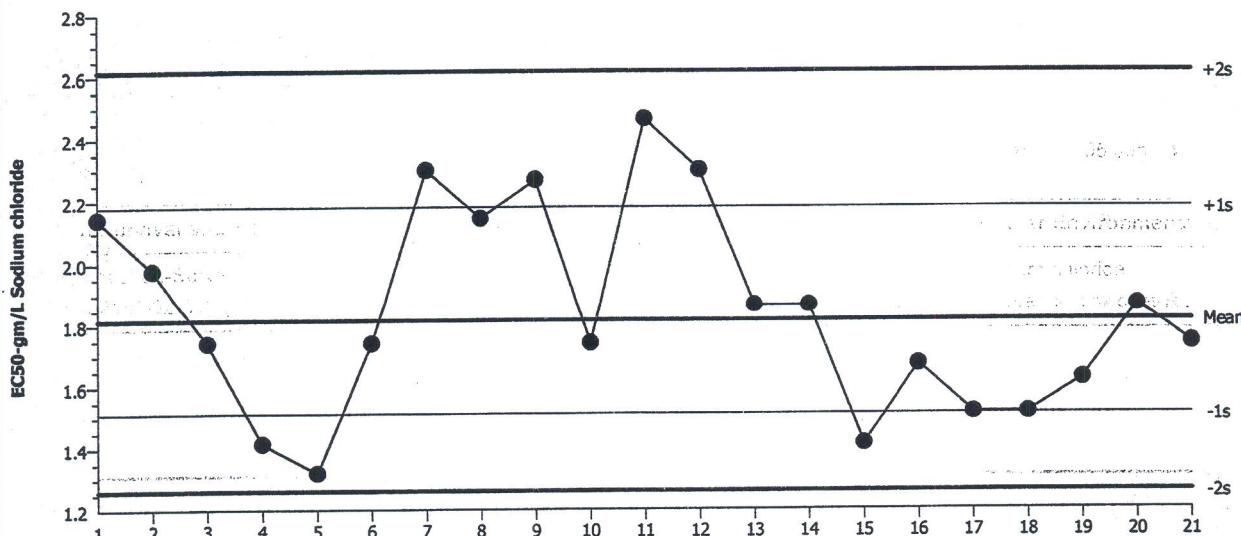
Report Date: 05 Jun-14 13:57 (1 of 1)

Ceriodaphnia 7-d Survival and Reproduction Test

Rainier Environmental Laboratory

Test Type: Reproduction-Survival (7d)
Protocol: EPA/821/R-02-013 (2002)Organism: Ceriodaphnia dubia (Water Flea)
Endpoint: 7d Survival RateMaterial: Sodium chloride
Source: Reference Toxicant-REF

Ceriodaphnia 7-d Survival and Reproduction Test



Mean: 1.815 Count: 20 -1s Warning Limit: 1.512 -2s Action Limit: 1.26
Sigma: NA CV: 20.00% +1s Warning Limit: 2.179 +2s Action Limit: 2.616

Quality Control Data

Point	Year	Month	Day	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2011	Nov	10	2.144	0.3282	0.91			18-1646-5148	10-8016-4521
2			18	1.977	0.1616	0.4671			11-7820-4865	00-4433-4101
3			19	1.741	-0.07429	-0.2288			08-1813-0818	01-3437-6850
4			25	1.414	-0.4012	-1.368	(-)		12-3676-9409	20-8924-6879
5		Dec	2	1.32	-0.4959	-1.747	(-)		03-1806-9758	03-8081-7233
6	2012	Mar	20	1.741	-0.07429	-0.2288			04-4181-4802	06-8447-2630
7		Jun	26	2.297	0.482	1.29	(+)		18-3335-1100	10-1106-6325
8		Jul	17	2.144	0.3282	0.91			02-9547-9197	16-5989-6607
9		Sep	18	2.267	0.4512	1.216	(+)		20-3257-9401	11-6459-7205
10		Oct	30	1.741	-0.07429	-0.2288			14-7011-9138	01-0378-8759
11		Dec	11	2.462	0.6469	1.669	(+)		18-7111-4230	17-4716-3730
12	2013	Mar	12	2.297	0.482	1.29	(+)		13-9507-4728	11-5347-8239
13		Jun	18	1.866	0.05068	0.1508			19-2937-3586	14-8537-9739
14		Jul	23	1.866	0.05068	0.1508			05-1063-7728	11-1141-7863
15		Sep	17	1.414	-0.4012	-1.368	(-)		04-6344-2666	12-0321-1244
16		Oct	8	1.672	-0.1436	-0.4513			19-4506-7718	00-1302-6176
17		Nov	5	1.516	-0.2997	-0.988			11-3928-9312	04-5813-4583
18		Dec	10	1.516	-0.2997	-0.988			18-7027-0129	20-1597-0849
19	2014	Jan	28	1.625	-0.1909	-0.6084			07-5179-7310	07-6531-8387
20		Mar	11	1.866	0.05068	0.1508			01-9460-5556	18-5161-8624
21		May	20	1.741	-0.07429	-0.2288			01-8746-7556	12-7653-6000

Ceriodaphnia 7-d Survival and Reproduction Test

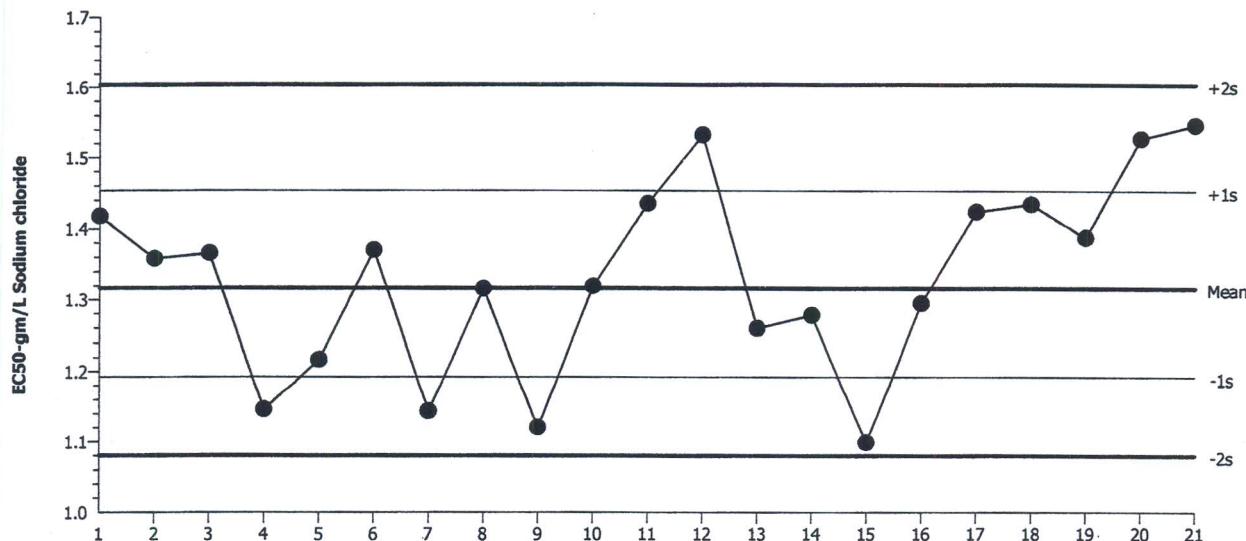
Rainier Environmental Laboratory

Test Type: Reproduction-Survival (7d)
 Protocol: EPA/821/R-02-013 (2002)

Organism: Ceriodaphnia dubia (Water Flea)
 Endpoint: Reproduction

Material: Sodium chloride
 Source: Reference Toxicant-REF

Ceriodaphnia 7-d Survival and Reproduction Test



Mean: 1.317 Count: 20 -1s Warning Limit: 1.193 -2s Action Limit: 1.081
 Sigma: NA CV: 10.40% +1s Warning Limit: 1.453 +2s Action Limit: 1.604

Quality Control Data

Point	Year	Month	Day	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2011	Nov	10	1.417	0.1009	0.7477			18-1646-5148	02-8001-5569
2			18	1.358	0.04149	0.3143			11-7820-4865	14-3007-5816
3			19	1.366	0.04936	0.3729			08-1813-0818	00-3657-8188
4			25	1.146	-0.1704	-1.404	(-)		12-3676-9409	18-2334-7627
5		Dec	2	1.216	-0.1	-0.8006			03-1806-9758	11-1567-8111
6	2012	Mar	20	1.37	0.05372	0.4051			04-4181-4802	14-9819-7042
7		Jun	26	1.144	-0.1727	-1.424	(-)		18-3335-1100	06-3063-9294
8		Jul	17	1.316	-0.00027	-0.00209			02-9547-9197	03-2978-8518
9		Sep	18	1.121	-0.1959	-1.632	(-)		20-3257-9401	21-2717-5233
10		Oct	30	1.319	0.00293	0.02253			14-7011-9138	13-0927-4963
11		Dec	11	1.435	0.1187	0.8745			18-7111-4230	00-5310-2281
12	2013	Mar	12	1.533	0.2165	1.542	(+)		13-9507-4728	19-0889-8785
13		Jun	18	1.26	-0.05666	-0.4456			19-2937-3586	04-7875-5639
14		Jul	23	1.278	-0.03813	-0.2977			05-1063-7728	12-7480-7387
15		Sep	17	1.099	-0.2171	-1.825	(-)		04-6344-2666	19-1590-3186
16		Oct	8	1.296	-0.02099	-0.1628			19-4506-7718	02-9851-4215
17		Nov	5	1.424	0.1075	0.7953			11-3928-9312	03-0064-1230
18		Dec	10	1.434	0.118	0.8692			18-7027-0129	07-4046-3167
19	2014	Jan	28	1.388	0.07167	0.5369			07-5179-7310	11-7115-8476
20		Mar	11	1.528	0.2112	1.507	(+)		01-9460-5556	11-2770-3964
21		May	20	1.547	0.2306	1.635	(+)		01-8746-7556	02-2261-6576

CETIS Summary Report

Report Date: 05 Jun-14 13:58 (p 1 of 2)
 Test Code: RT052014CD | 01-8746-7556

Ceriodaphnia 7-d Survival and Reproduction Test

Rainier Environmental Laboratory

Batch ID:	17-9283-7000	Test Type:	Reproduction-Survival (7d)	Analyst:	Eric Tollefson
Start Date:	20 May-14 14:15	Protocol:	EPA/821/R-02-013 (2002)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	27 May-14 14:45	Species:	Ceriodaphnia dubia	Brine:	
Duration:	7d 1h	Source:	In-House Culture	Age:	<24h
Sample ID:	15-4881-9333	Code:	RT052014CD	Client:	Internal Lab
Sample Date:	20 May-14	Material:	Sodium chloride	Project:	
Receive Date:	20 May-14	Source:	Reference Toxicant		
Sample Age:	14h	Station:	In House		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
16-0284-3974	7d Survival Rate	2	4	2.828	NA		Fisher Exact/Bonferroni-Holm Test
08-5709-9853	Reproduction	0.5	1	0.7071	18.7%		Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	gm/L	95% LCL	95% UCL	TU	Method
12-7653-6000	7d Survival Rate	LC50	1.741	1.315	2.305		Spearman-Kärber
02-2261-6576	Reproduction	IC5	0.5215	0.1901	0.6113		Linear Interpolation (ICPIN)
		IC10	0.61	0.4142	0.7656		
		IC15	0.7037	0.546	0.9493		
		IC20	0.8028	0.633	1.074		
		IC25	0.9077	0.7111	1.183		
		IC40	1.268	0.9323	1.565		
		IC50	1.547	1.192	1.915		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
12-7653-6000	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
16-0284-3974	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
02-2261-6576	Reproduction	Control Resp	19.9	15 - NL	Yes	Passes Acceptability Criteria
08-5709-9853	Reproduction	Control Resp	19.9	15 - NL	Yes	Passes Acceptability Criteria
08-5709-9853	Reproduction	PMSD	0.1874	0.13 - 0.47	Yes	Passes Acceptability Criteria

7d Survival Rate Summary

C-gm/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	1	1	1	1	1	0	0	0.0%	0.0%
0.25		10	1	1	1	1	1	0	0	0.0%	0.0%
0.5		10	1	1	1	1	1	0	0	0.0%	0.0%
1		10	0.8	0.6426	0.9574	0	1	0.1333	0.4216	52.7%	20.0%
2		10	0.5	0.3032	0.6968	0	1	0.1667	0.527	105.4%	50.0%
4		10	0	0	0	0	0	0	0		100.0%

Reproduction Summary

C-gm/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	19.9	19.01	20.79	15	23	0.752	2.378	11.95%	0.0%
0.25		10	20.2	19	21.4	12	24	1.02	3.225	15.96%	-1.51%
0.5		10	19.3	18.4	20.2	15	23	0.7608	2.406	12.47%	3.02%
1		10	14.2	12.13	16.27	0	19	1.75	5.534	38.97%	28.64%
2		10	7.2	5.618	8.782	0	13	1.34	4.237	58.85%	63.82%
4		10	0	0	0	0	0	0	0		100.0%

CETIS Summary Report

Report Date:

05 Jun-14 13:58 (p 2 of 2)

Test Code:

RT052014CD | 01-8746-7556

Ceriodaphnia 7-d Survival and Reproduction Test**Rainier Environmental Laboratory****7d Survival Rate Detail**

C-gm/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1	1	1	1	1	1	1	1	1	1
0.25		1	1	1	1	1	1	1	1	1	1
0.5		1	1	1	1	1	1	1	1	1	1
1		1	1	1	1	0	1	1	0	1	1
2		1	0	1	0	1	0	1	0	0	1
4		0	0	0	0	0	0	0	0	0	0

Reproduction Detail

C-gm/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	23	17	20	20	21	15	22	21	21	19
0.25		22	20	20	20	12	21	19	24	22	22
0.5		23	17	18	20	20	19	18	21	22	15
1		17	16	15	19	10	15	16	0	18	16
2		13	8	12	5	12	3	6	5	0	8
4		0	0	0	0	0	0	0	0	0	0

7d Survival Rate Binomials

C-gm/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.25		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.5		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
1		1/1	1/1	1/1	1/1	0/1	1/1	1/1	0/1	1/1	1/1
2		1/1	0/1	1/1	0/1	1/1	0/1	1/1	0/1	0/1	1/1
4		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

Rainier Environmental
Washington Laboratory

Client: Reference Testicort
Sample ID: 4g/L NaCl
Test No: RT052014CD
Log-In#: —

Initial and Final Chemistries

Seven Day Chronic Freshwater Bioassay

Start Date & Time: 5/20/14 1415

Stop Date & Time: 5/27/14 1445

Test Species: Ceriodaphnia dubia

Conc. or % CON	Days													
	0		1		2		3		4		5		6	
	init.	final												
pH	7.51	7.68	7.55	7.38	7.71	7.98	7.59	7.91	7.62	8.02	7.59	8.05	7.65	7.98
DO (mg/l)	7.9	8.2	8.0	8.2	8.1	8.3	7.9	8.2	8.0	8.2	7.9	8.2	7.9	8.2
Cond. (μmhos-cm)	195	201	195	198	196	204	197	208	197	211	195	204	196	203
Temperature (°C)	24.2	25.5	24.5	25.2	24.8	25.2	25.2	25.5	24.9	25.3	25.1	25.2	24.4	25.2
0.25	Days													
	0		1		2		3		4		5		6	
	init.	final												
pH	7.50	7.67	7.55	7.45	7.69	7.95	7.58	7.91	7.63	8.04	7.57	8.01	7.62	7.99
DO (mg/l)	7.8	8.2	8.1	8.2	8.1	8.2	7.8	8.2	7.9	8.2	7.7	8.2	8.1	8.2
Cond. (μmhos-cm)	597	612	601	601	595	601	603	613	600	608	594	599	591	595
Temperature (°C)	24.2	25.5	24.6	25.2	24.8	25.2	25.3	25.5	25.0	25.3	25.1	25.2	24.4	25.2
0.5	Days													
	0		1		2		3		4		5		6	
	init.	final												
pH	7.51	7.71	7.55	7.47	7.69	7.95	7.58	7.92	7.60	8.01	7.59	8.01	7.62	7.95
DO (mg/l)	7.8	8.2	8.1	8.2	8.0	8.2	7.9	8.2	7.9	8.2	7.9	8.2	8.1	8.2
Cond. (μmhos-cm)	1183	1185	1203	1198	1192	1201	1197	1204	1205	1215	1207	1213	1211	1212
Temperature (°C)	24.2	25.5	24.6	25.3	24.8	25.2	25.3	25.5	25.0	25.3	25.2	25.2	24.4	25.2
1	Days													
	0		1		2		3		4		5		6	
	init.	final												
pH	7.51	7.68	7.55	7.49	7.69	7.94	7.57	7.93	7.60	8.01	7.59	8.07	7.63	7.97
DO (mg/l)	7.8	8.2	8.0	8.2	7.9	8.2	7.8	8.2	7.8	8.2	7.9	8.2	8.1	8.2
Cond. (μmhos-cm)	2231	2242	2228	2247	2213	2231	2198	2208	2238	2243	2215	2219	2221	2232
Temperature (°C)	24.2	25.5	24.6	25.3	24.8	25.2	25.3	25.5	25.0	25.3	25.3	25.2	24.5	25.2
2	Days													
	0		1		2		3		4		5		6	
	init.	final												
pH	7.52	7.69	7.55	7.51	7.66	7.92	7.57	7.93	7.61	8.02	7.60	8.08	7.63	7.98
DO (mg/l)	7.8	8.2	7.9	8.2	7.9	8.2	7.7	8.2	7.8	8.2	7.9	8.2	8.0	8.2
Cond. (μmhos-cm)	3900	3910	3880	3860	3920	3920	3920	3940	3900	3920	3890	3920	3870	3870
Temperature (°C)	24.2	25.5	24.4	25.3	24.8	25.2	25.3	25.5	25.0	25.3	25.2	25.2	24.5	25.2
4	Days													
	0		1		2		3		4		5		6	
	init.	final												
pH	7.52	7.69												
DO (mg/l)	7.8	8.2												
Cond. (μmhos-cm)	7420	7420												
Temperature (°C)	24.2	25.5												
Tech. Initials	8+	8+	9+	8+	9+	9+	9+	9+	8+	8+	8+	8+	8+	8+

Dilution Water Batch #: DMW004

Test Chamber: VWR

OA Check:

七

Sample Description:

Animal Source:

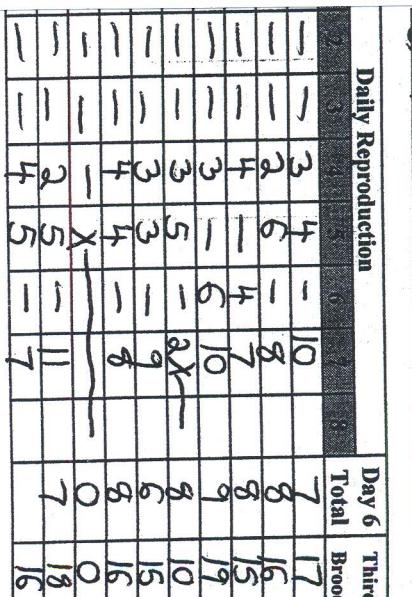
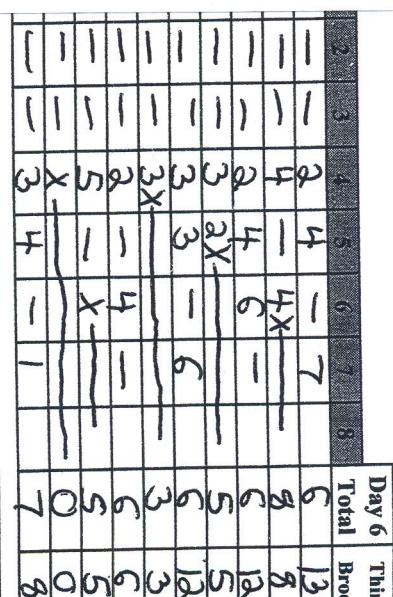
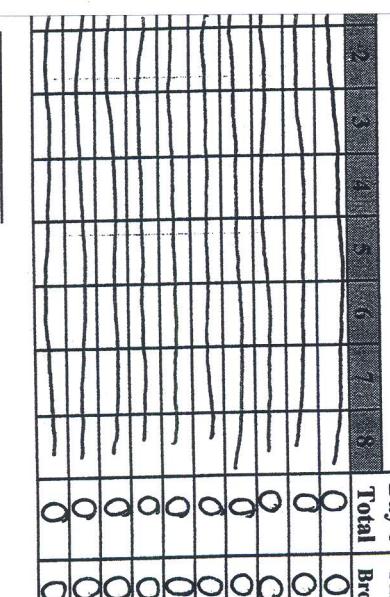
Comments:

4g/L NaCl

In House culture

Date Received:

Date of Hatch:



Rainier ENVIRONMENTAL

Washington
 5013 Pacific Highway East, Suite 20 Fife,
 WA 98424
 Phone 253.922.8898

Date 6/4/14 Page 1 of 1

Sample Collection By:

Report to: Washington Beef L.L.C.
Company 201 7th Ave. Rd.
Address Tacoma, WA. 98448
City/State/Zip
Contact Sherri Byers
Phone 509 4952-6534
Email Sherri.Byers@abfoods.usg.com

Invoice To:

Company
Address
City/State/Zip
Contact Same
Phone
Email

ANALYSES REQUIRED

Receipt Temperature (°C)

SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	NO. OF CONTAINERS	COMMENTS	
1 NET	6/4/14	7:25AM	Master Box	Cubitainer	1	X	
2							
3							
4							
5							
6							
7							
8							
9							
10							
PROJECT INFORMATION							
SAMPLE RECEIPT				RELINQUISHED BY (CLIENT)		RELINQUISHED BY (COURIER)	
Client:	Total No. of Containers	(Signature)	(Time)	(Signature)	(Time)	(Signature)	(Time)
PO No.:	1	<u>H. M. Richardson</u>	<u>11:40 AM</u>	<u>Eric Tolleson</u>	<u>12:05</u>	<u>Eric Tolleson</u>	<u>12:05</u>
Shipped Via:	UPS	(Printed Name)	(Date)	(Printed Name)	(Date)	(Printed Name)	(Date)
SPECIAL INSTRUCTIONS/COMMENTS:							
RECEIVED BY (COURIER)				RECEIVED BY (LABORATORY)			
(Signature)		(Time)	(Signature)	(Time)		(Signature)	
(Printed Name)		(Date)	(Printed Name)	(Date)		(Printed Name)	
ERIC TOLLESON							
6/5/14							
14-058							

Rainier ENVIRONMENTAL

Washington
5013 Pacific Highway East, Suite 20 Fife,
WA 98424
Phone 253.922.8898

Date 6/2/14 Page 1 of 1

Chain of Custody

Sample Collection By:

Report to:
Company Washington Beef LLC
Address 201 Elmwood Rd
City/State/Zip Toppenish WA 98948
Contact Sherry Byers
Phone 509.952-6534
Email Sherry.Byers@abfounds.usa.com

Invoice To:

Company
Address
City/State/Zip
Contact
Phone
Email

ANALYSES REQUIRED

Receipt Temperature (°C)

SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	NO. OF CONTAINERS	COMMENTS		ANALYSES REQUIRED
1	6/2/14	8:00	Waste Water	Cubefamer	1	X	A	WT
2								
3			Pine Chest					
4								
5								
6								
7								
8								
9								
10								

PROJECT INFORMATION		SAMPLE RECEIPT		RELINQUISHED BY (CLIENT)		RELINQUISHED BY (COURIER)	
Client:	PO No.:	Total No. of Containers	Received Good Condition?	(Signature) 	(Time) 11:30AM	(Signature) 	(Time)
				(Printed Name) Bonnie M. Harris-Richardson	(Date) 6/2/14	(Printed Name)	(Date)
Shipped Via:	Via:	Matches Test Schedule?		(Company) Washington Beef LLC	(Company)		

RECEIVED BY (COURIER)		RECEIVED BY (LABORATORY)	
(Signature)	(Time)	(Signature)	(Time)
(Printed Name)	(Date)	(Printed Name)	(Date)
(Company)		(Company)	
ERIC TOLLEFSON	6/3/14	ERIC TOLLEFSON	14-056